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A STUDY OF THE REACTION OF MESENCHYME CELLS IN THE TAD-POLE'S TAIL TOWARD INJECTED OIL GLOBULES[1]

ELIOT R. CLARK

From the Anatomical Laboratory of the University of Missouri

FIVE FIGURES

In their later studies on the mode of development of the lymphatic system, Huntington and McClure have reiterated the view that the lymphatics are formed by the transformation of mesenchyme cells in the following manner. They hold that fluid accumulates in the tissue spaces, forming small lakelets; that the mesenchyme cells are pressed upon by the fluid collected; and that, as a result of this mechanical pressure, the mesenchyme cells react by flattening out and by forming membranes around the blisters.

The main evidence presented in favor of this view consists in the finding, in microscopic sections, of clear spaces in the tissues, unsurrounded by any membrane, of other spaces which have the appearance of being partly surrounded, and of others with a complete covering. Some of these appear to be completely isolated while others are connected with one another. The numerous possibilities of error in the interpretation of the appearances described have been pointed out by Miss Sabin (1), E. L. Clark (2), and myself (3), and will not be reviewed at this time.

The importance laid by these investigators on the part played by the mechanical action of fluid on mesenchyme cells may be

[1] A preliminary report of these studies was published in the Proceedings of the American Ass. of Anat., Anat. Rec., vol. 10, no. 3, 1916, p. 191.

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seen from the following quotation. Huntington (4) says (page 289):

> If two embryonal mesenchymal cells are separated from each other by the accumulation of fluid in the resulting intercellular space, then the opposing aspects of the two cells involved will be subjected to the mechanical and hydrostatic influences of accumulated intercellular fluid, which will react upon the surfaces of the cell still held in syncytial relation to the surrounding mesenchyme. The cells whose opposing surfaces have become freed by the development of an intercellular space, and are subjected to fluid pressure, will react as a whole, become flattened, and be transformed into endothelial cells, forming the parietal limit of an originally intercellular mesenchymal space, which is the font and origin of all vertebrate vascular development.

This should be supplemented by a quotation from McClure (5), for no explanation is given here for the accumulation of fluid in the tissues.

> As soon, however, as the haemal vessels begin to function, lymph begins to collect in the intercellular spaces of the embryo and, as we know, is subsequently collected by a set of newly formed vessels, the lymphatics, which convey it to the venous circulation.
>
> Those who maintain that the lymphatics sprout centrifugally and continuously from the veins, would necessarily hold that the lymph in the intercellular spaces patiently awaits the arrival of close and hollow outgrowths from the veins, the lymphatics, before it can be received into any portion of the lymphatic system.

The combined picture, then, is as follows: as soon as the blood-vessels function, "lymph begins to collect in the intercellular spaces." It collects apparently because it cannot get back into the blood-vessels. The mesenchyme cells are subjected to "mechanical and hydrostatic pressure" exerted by the accumulated lymph, and respond by flattening out, and becoming endothelial cells. This comprises the "font and origin of all vertebrate vascular development."

Some of the theoretical objections to this series of assumptions may be pointed out.

First, McClure states that "as soon as the haemal vessels begin to function, lymph begins to collect in the intercellular spaces of the embryo;" to the pressure exerted by this accumulated lymph is assigned the rôle of acting as the formative

stimulus for the transformation of mesenchyme cells into lymphatics. In another place, McClure argues that blood-vessels and lymphatics differentiate in the same manner. Now if the collection of intercellular lymph begins only after the blood-vessels begin to function, how are we to explain the collections of fluid which are supposed to have served as a formative stimulus for the differentiation of blood-vessels? Again, if both blood-vessels and lymphatics are determined solely by the action of mechanical pressure, we are left with the same puzzling problem which confronted Goette (6) in 1873, namely, why the two sets of vessels do not everywhere form communications with one another. The puzzle differs only in that Goette conceived of the circulation from blood to lymph capillary as being intracellular, and tried to explain why the same mesenchyme cell was not sometimes demanded by both the blood-vessel and the lymphatic, while Huntington and McClure conceive of the fluid as being extra-cellular and must explain why it does not happen that the same lakelet does not connect with a blood capillary on the one side, and with a lymphatic capillary on the other. Goette's puzzle has been eliminated by the proof that in the tail of amphibian larvae, mesenchyme cells take no part in the growth of blood or lymphatic vessels.

Again, it is hardly necessary that "those who maintain that the lymphatic sprout centrifugally and continuously from the veins, would necessarily hold that the lymph in the intercellular spaces patiently awaits the arrival of closed and hollow outgrowths from the veins, the lymphatics," since it was demonstrated clearly by Magendie, over a hundred years ago, and has been proven so many times since that it cannot be questioned, that absorption of substances may take place through the blood-vessels as well as through the lymphatics.

Another assumption which is quite unwarranted on the basis of facts is that with an increase in intercellular fluid, this fluid will collect in definite lakelets, in the intercellular spaces. All our knowledge of intercellular spaces indicates that they form an irregular, intercommunicating net-work of spaces filled with fluid. It would be expected that, if this fluid were

increased, the increase in the intercellular spaces would be a general one, the distention, or edema, being regulated in its intensity only by the relative amount of resistance offered by the tissue. This resistance would, of course, be greater in dense tissues, in which the cells are bound firmly to one another, and less in tissues where cells are less firmly attached to one another. In any region, however, where the tissue is uniform the pressure and separation of cells must be uniform, and no accumulation of tissue fluid in the form of lakelets would seem to be possible. Microscopic examination of embryos bears out this theoretical consideration; there are regions in which the intercellular fluid is large in amount, proportionately, and others where it is small. Where large, the mesenchyme or other cells are uniformly separated from one another, as for example in the umbilical cord, and the ventral body wall.

It is unjustifiable from our knowledge, or better, lack of knowledge, to speak positively about the stimuli which are responsible for the primary differentiation of organs or tissues, since with, perhaps, the exception of the formation of the lens as the result of the contact of the optic cup with the epidermis (7), there is hardly an instance in embryology of the satisfactory demonstration of the stimulus responsible for the primary differentiation of any organ or tissue (8). In this connection, results obtained in recent studies made on tissues, grown by the 'tissue culture' method of Harrison, are interesting. Harrison (9) found that primitive nerve cells send out processes into a medium consisting of coagulated lymph. The Lewises (10) obtained similar results with sympathetic nerve cells, in a medium of Locke's solution. Shipley (11) has found that undifferentiated heart muscle cells differentiate and start rhythmic contractions, in a medium of coagulated plasma. In all of these cases the cells were removed from their normal environment. Their continued development makes one sceptical of hypotheses as to the nature of formative stimuli, when such hypotheses are not supported by fact.

The only experimental evidence which has been proposed in support of the hypothesis that collections of lymph furnish

the stimulus to the formation of lymphatics, consists of the results of studies on 'experimental mesothelium,' by W. C. Clark (12). This investigator found that if solid blocks of celloidin are placed in the subcutaneous tissue of dogs, they became surrounded by flattened cells, which show, when treated with silver salts, black intercellular lines typical of flattened mesothelial or epithelial tissues. Solid globules of hard paraffin, injected into the cornea of rabbits, were surrounded by a layer of flattened cells. Flattened cells were also found to line 'dead spaces' in the tissue and artificial channels, such as may be induced by the ligation of the cystic duct, with formation of a mucous fistula.

Interesting as are these studies, in spite of the absence of evidence as to the source of origin of the cells which formed the flattened lining membrane, there appears to be no justification for the conclusion that we have thereby gained any information as to the mode of differentiation or growth of blood or lymphatic vessels. And yet, W. C. Clark concludes (page 316) that "Therefore the second hypothesis, premised in this article, is tenable, namely that the flat cells of serous surfaces and those lining blood vessels may regenerate from deep connective tissue cells, and do not necessarily arise from adjacent intact mesothelial or endothelial cells." Again McClure (13) refers to the results of W. C. Clark as bearing out "in a most decisive manner" the view that "the gradual increase in the amount of lymph received by the subocular sacs (in the trout) during the stage of their independence, results in the application of a constant and continuous pressure to the mesenchyme cells forming their walls, which in itself must be a positive factor in causing these cells to flatten out and gradually assume an endothelial form."

It is difficult to conceive how the results obtained by W. C. Clark can have any bearing on the problem which confronts McClure, unless the assumption is made that all mesothelial, endothelial, and epithelial membranes which line spaces or ducts have the same properties—an assumption for which facts furnish no justification. Surely blood-vessel endothelium has

properties which differ from synovial membranes, peritoneal membranes, or the lining membrane of the urethra, the bile ducts, or the gall-bladder.

There is, perhaps, a suggestion of support for the hypothesis in question in some of the results obtained in tissue cultures. Several observers—Harrison (14), M. R. and W. H. Lewis (15), Lambert (16), W. C. Clark (17) and others—have found that in bits of tissues, explanted to plasma or Locke's solution, membranes may be formed around solid bodies—such as along the cover-slip and around solid threads, as the threads of spider web, used by Harrison, and around droplets of fluid—such as may be formed, occasionally, in plasma preparations, by the retraction of the fibrous threads. The explanation of the formation of membranes around droplets of fluid is associated with the apparent inability of cells to grow into a purely fluid medium, without mechanical support, or as expressed by Harrison, their dependence on 'stereotropism.' The formation of such a membrane in tissue cultures is not to be interpreted as a reaction by flattening out, on the part of the culture cells, but rather, in all probability, as due to the fact that cells grow around the periphery of such a droplet. Moreover, it has not yet been possible to determine the origin of the cells which form membranes in tissue cultures. At present there appears to be no ground for claiming that the formation of membranes in this manner, furnishes us any information as to the mode of differentiation of blood-vessel or lymphatic endothelium.

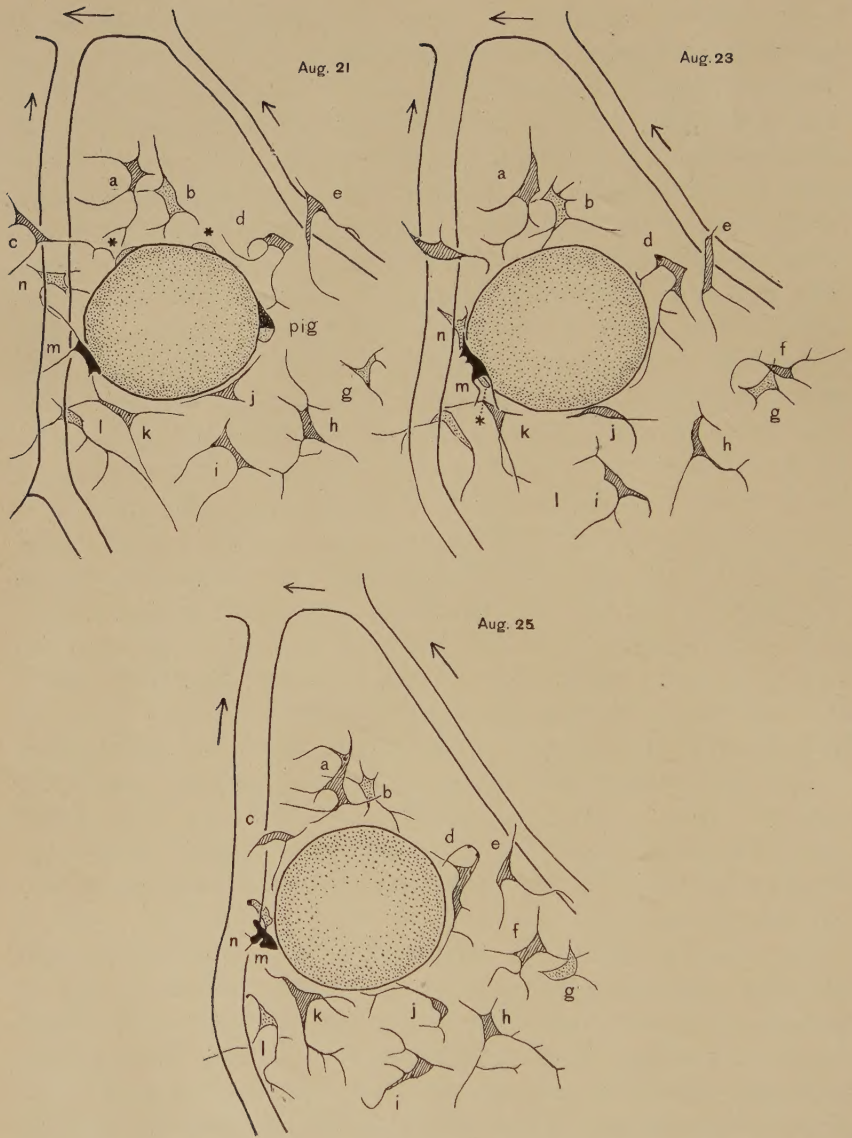
In order to put to the test the hypothesis that the differentiation of blood or lymph vessel endothelium may be stimulated by the mechanical pressure exerted on mesenchyme cells by accumulations of fluid, and to plan the test in such a way as to make it approach as nearly as possible the actual conditions supposed to exist, the present studies were started. The aim was to inject an inert fluid, in globules of size sufficient to press against the mesenchyme cells, into a region of embryonic tissue, in which the reaction of the cells could be watched in the living animal; to see whether a membrane were formed, and, if so, by what type of cell, and what would be the properties of such a

membrane, especially its reaction toward blood-vessel and lymphatic endothelium.

In order to simulate as nearly as possible the fluid whose presence is thought to excite the transformation of mesenchyme cells into lymphatics, and at the same time to have a fluid which would be inert, which would not be absorbed, which would merely exert a mechanical pressure, paraffin oil was selected for injection. The object chosen was the transparent fin expansion of the tail of young frog and toad tad-poles where it is possible to see the individual mesenchyme cells, as well as blood-vessels, lymphatics and leucocytes, and to watch their reactions in the living larvae, from day to day. The tad-poles were anaesthetized with chloretone (1:4000 to 1:5000) and small globules of oil injected into both fins, through fine glass cannulae, under the binocular microscope. The oil was sterilized by heating. In some cases the tadpole was washed in several changes of sterile water, but the results did not differ materially from those obtained when the only antiseptic precaution consisted in sterilizing the oil. The observations were made by a method previously described in detail (18)—the larva, anesthetized, was placed in a micro-aquarium, in chloretone of the proper strength, and the tube of the microscope tilted to the horizontal, to enable the tadpole to retain its normal upright position. The oil globules were of varying sizes, from 20 to 100 micra in diameter.

The larvae used were those of Rana catesbiana (bull-frog) and of Fowler's toad (Bufo lentiginosus Fowleri). The latter have beautifully clear tails, with very few pigment cells at the stages used, while the mesenchyme cells are far apart and stand out most clearly.

Since the oil could not be injected without a certain amount of injury, there were always some temporary effects of the injection, not attributable to the presence of the oil. These consisted principally of a more or less intense leucocytosis, probably caused by greater or milder degrees of infection. In some cases large numbers of leucocytes gathered about the globules, many of the leucocytes containing pigment. Several of the globules, around which the leucocytosis was most intense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were extruded. In case this did not occur, the leucocytosis gradually subsided until in most cases three or four days after the injection, the region surrounding the globule contained no more leucocytes than the other parts of the tail. In some instances, a slightly increased number of leucocytes near the globule continued for several days longer. From now on the globules remained apparently inert, so far as could be judged from the behavior of leucocytes in their vicinity. The longest time over which a globule was watched was 12 days.

In appearance the oil globules, when present in the tail, form spheres with the central portion clear and transparent and a dark periphery, with a sharp outline. Structures over the central portion can be seen most clearly. Thus it is possible, in case the diameter of the globule is sufficient to distend the skin slightly, to see the nuclei of the cells of the epidermis, and the details of other structures most distinctly. Many of the globules were oval in shape, immediately after injection. Later, after a day or two, they usually rounded up to a spherical shape though sometimes remaining slightly oval.

The behavior of the mesenchyme cells will now be described. In order to follow them with accuracy camera lucida records were made of all mesenchyme cells in the neighborhood of the globules, and their changes from day to day were noted. When a mesenchyme cell happened to be in the outer dark zone of the globule, it was difficult to make out its outlines. Occasionally only one or two of its processes could be clearly seen. The following of such cells, however, was made possible by the fact

Fig. 1 From larva of Fowler's toad, 9 mm. long. Four globules of paraffin oil injected into fin expansion of tail, on Aug. 19. Much leucocytosis about three of them, and all three extruded within forty-eight hours. There was very little leucocytosis about the fourth globule, of which three drawings are shown, two, four, and six days after injection. This globule was in the ventral fin. The mesenchyme cells in the immediate vicinity of the globule are shown. The letters, *a*, *b*, etc., indicate the same cells. * leucocytes against the globule; *pig.L.*, pigmented leucocyte against the globule. The mesenchyme cells were in three different planes; those nearest the observer are represented in solid black, those furthest away are dotted, while those in the midst are cross-hatched. Enlarged 267 times. Drawn with camera lucida.

that the globules from time to time shifted their position slightly, so that a cell, at one time not clearly seen, later could be clearly outlined. This applied to only a very few cells, particularly in the toad larvae, because of the relative rarity of their mesenchyme cells. In view of the descriptions in the literature of the reaction of connective tissue cells to the pressure exerted by foreign substances, the behavior of the mesenchyme cells was a great surprise. It was expected that the cells near the globule would flatten out on its surface and form a membrane. On the contrary, the mesenchyme cells apparently paid no particular attention to the globules. They maintained their identity as 'star-shaped' cells, with thickened central portion and branched processes, and their property of slow progression, described in an earlier paper. That the mesenchyme cells are not influenced by the pressure exerted by the globules was brought out quite strikingly in one instance in which the globule shifted its position in such a way as to come to lie against a mesenchyme cell which had been at a slight distance from the globule. For a day or two it was rather difficult to make out the outlines of the cell. The globule then shifted its position in the opposite direction, and the mesenchyme cell could now be seen clearly, apparently unchanged, at a slight distance from the globule. Occasionally there are to be seen, over the clear part of the globule, if the globule is of sufficient size to distend the skin slightly, one or two flattened cells which, at first glance, might be interpreted as cells flattened out by the pressure of the globule. Such cells were seen over only a few of the globules, and their explanation was obvious on studying other parts of the tail, at a distance from the globules. Such flattened out mesenchyme cells appear more or ess evenly distributed, lying just below the epidermis, and are not particularly associated with the oil globules. That they are associated with the skin and not with the oil globules, is shown by the fact that none are present over the majority of the globules, and also by the fact that, if the globules shift, these cells maintain the same position with reference to the skin, while they are left behind by the oil globules (fig. 4). There are no other appearances on

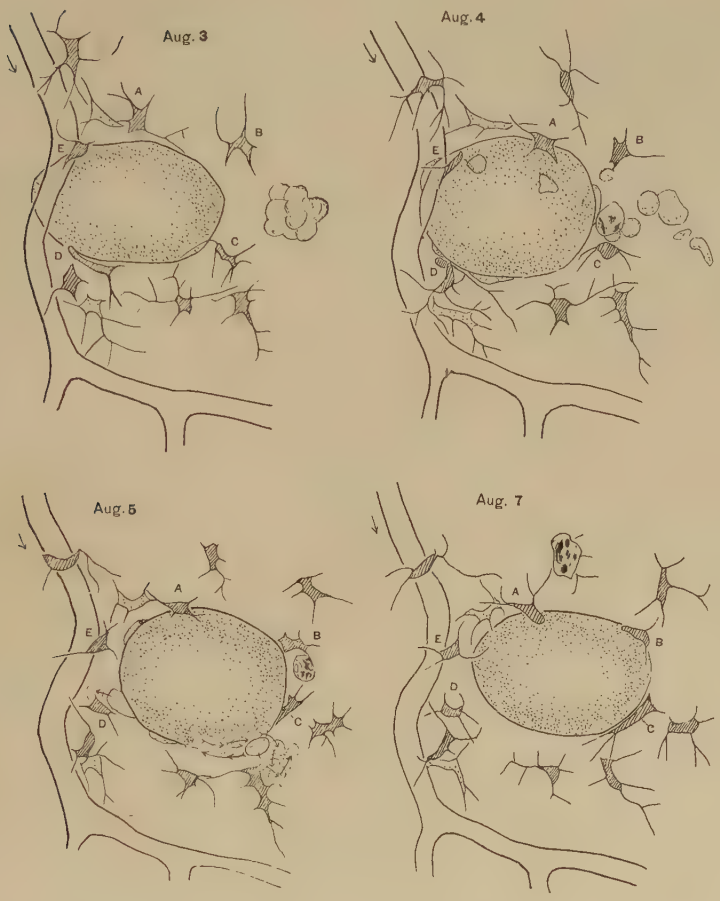

Fig. 2 From larva of Rana catesbiana, 10.5 mm. long. Two globules of paraffin oil injected into the ventral, and a small globule into the dorsal fin; all three remained. Much leucocytosis around each. The first drawing (Aug. 3) was made immediately after the injection. Small mass of cellular débris is shown, in the path of the injection. Mesenchyme nearest the globule lettered as in fig. 1. In drawings Aug. 4, 5, and 7, some of the leucocytes about the globule are shown. In drawing of Aug. 5 are shown the successive positions taken by a leucocyte as it moved to the globule, moved along the surface of the globule a short distance, and then moved away. The shifting of the globule is clearly seen, by comparing its relation to the blood vessel. The cells *d* and *e*, which lie close to the globule Aug. 3 and 4, are left behind, while the cells *b* and *c* are approached by the shifting of the globule.

Enlarged 267 times. Drawn with camera lucida.

the part of the mesenchyme cells which even remotely suggested a flattening out. The fact that some of the globules watched shifted their position would also indicate that no surrounding membrane had been formed, for a membrane would prevent such movement.

The behavior of wandering cells towards the globules was watched with interest. As already stated there was a leucocytosis of greater or less intensity following the introduction of the globules, for the first two or three days. Leucocytes, most of them small and clear, others larger, and containing pigment, collected around the globules. Many of them flattened themselves out on the surface of the globule, or formed irregular humps on the profile. Occasionally such a flattened leucocyte formed a thin, circular structure, with nucleus visible over the clearest, central portion of the globule. Such a cell, coupled with the irregular humps on the profile, if seen only at one stage, and not followed, might well give the impression of membrane formation by wandering cells. When, however, such cells were followed, it was seen that they gradually moved away (fig. 2). The humps on the profile changed shape, with each drawing, even when the records were made several times daily, while the flattened cells on the clearer part moved away. After four or five days most of the globules were quite free from the presence of leucocytes. In order to be sure of the behavior of the wandering cells, some were watched intensively. They were seen to move, with the typical amoeboid type of progression, up to the oil globule, to flatten themselves out on its surface and again move away. The impression was gained, that, had sections been made at a time when the leucocytes were flattened on the globule, they might have been interpreted as forming a membrane, an interpretation which the study of the living shows would be quite unjustifiable.

Chromatophores which are present in large numbers in the dorsal fins of bull-frog larvae and to a somewhat less extent in the ventral fins of the same larvae, sometimes wrapped around the globules with their long branched processes, when the glob-

ules were injected near them, but did not form a definite membrane.

Since the mesenchyme cells failed to form a membrane around the globules, the second part of the inquiry, namely, the reaction of such a membrane, if formed, toward the lymphatics or blood-vessels in their vicinity, could not be followed. It was of interest, however, to observe the reaction of blood-vessel and lymphatic endothelium to the oil globules. In one case a particularly favorable opportunity was afforded to study the reaction of the blood-capillary, since the globule pressed against a blood-capillary, forcing it to make a bend in its course (fig. 4).

Fig. 3 From same Rana catesbiana larva from which figure 2 was taken. To show relation of pigment cells to globule. This small oil globule was injected into the dorsal fin, on Aug. 3; the drawing was made Aug. 5.
Enlarged 267 times. Camera lucida.

The capillary then appeared to wrap around a part of the globule. In this position the capillary showed no tendency to give off cells which might grow around the globule, but instead remained as a distinct vessel. The circulation of blood cells through it, which was at first interrupted, was later resumed. Blood-capillaries and lymphatics near the oil globules showed no tendency to grow toward it, or to send out sprouts to it.

The results of this study, then, indicate that, aside from the temporary inflammatory reaction, due probably to the injury and to the bacteria introduced at the time of injection, the presence in the fin of the tad-pole's tail of injected globules of paraffin oil, of sufficient size to cause a distension of the tissues, fails

to stimulate the formation of membranes about the globules, on the part of mesenchyme cells, wandering cells, or of blood-vessel or lymphatic endothelium.

These results are in disagreement with those of W. C. Clark (19), already mentioned. They are, however, in agreement with the older findings of E. Juckuff (20) who found that soft paraffin, injected subcutaneously, travelled long distances from the injection site, showing that no membrane was formed about

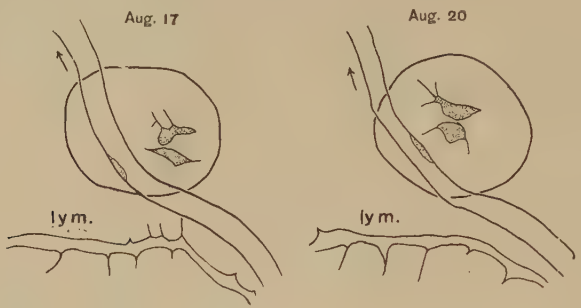

Fig. 4 Globule of paraffin oil injected into ventral fin of tail of Rana catesbiana larva on Aug. 13. The globule rested against a blood-capillary, forcing it to bend slightly. The two sketches shown, made four and seven days respectively after the injection, show the relation of the globule to the blood capillary, to a nearby lymphatic, (*lym*) and to two mesenchyme cells which happened to be under the epidermis immediately over the globule. Note that the globule has shifted to the right, so that the two mesenchyme cells, which on Aug. 17 are over the right part of the globule, are over the left central portion Aug. 20. Other mesenchyme cells not shown.

Enlarged 267 times. Drawn with camera lucida.

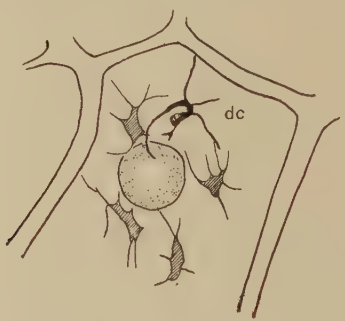

Fig. 5 Same larva as figure 4. Small globule injected in dorsal fin on Aug. 13. Drawing made nine days later—on Aug. 22, *d.c.*, pigment cell. Enlarged 267 times. Drawn with camera lucida.

it—a finding verified by MacCallum (21). It will be remembered that the attempt was made, in selecting the substance to be injected, to find something which would simulate the supposed lakelets of tissue fluid whose presence are held by Huntington and McClure to stimulate, merely by the mechanical pressure which they are supposed to exert, the mesenchyme cells to form membranes. It is obvious that in point of size and consistency the small globules of oil much more nearly reproduce the supposed conditions than the relatively enormous pus pockets, or the relatively huge solid blocks of celloidin or the globules of hard paraffin. It is also obvious that in a transparent object, like the tad-pole's tail, where the individual cells may be seen with great clearness and the reaction process watched in the living animal, the conditions for observing what happens are much more favorable than in the case of the other experiments referred to.

Since, then, the action of pressure alone fails to stimulate the formation of membranes on the part of mesenchyme cells, an important link in the argument used in favor of the origin of lymphatics from mesenchyme cells, as presented by Huntington and McClure, drops out.

A certain feeling of disappointment must be confessed, that the mesenchyme cells failed to respond to the presence of the oil globules by the formation around them of membranes, as it was expected they would, because of the desire to see what would be the reaction of such membranes toward blood-vessels and lymphatics.

It is true that cells derived from the middle layer or mesoderm differentiate at various stages into pavement epithelium, or endothelium, other than that which lines the blood and lymph vascular systems. Among such may be mentioned the lining of the large cavities pleural, peritoneal, and pericardial, the lining of bursae and synovial membranes, and the outer layers of tendons and fasciae. It should also be remembered that the same middle embryonic layer differentiates into smooth and striated muscle, into cartilage and bone, into blood cells, and other types of tissues. Each of these tissues has certain

modes of reaction, a specific life history, the property of responding each in its own individual way, to various stimuli. To transfer the modes of reaction of one set of tissues derived from the mesoderm to another set is quite unjustifiable. To be more specific, it is not justifiable to claim that, if connective tissues, in adult animals, are capable of forming membranes about solid foreign bodies, or large accumulations of fluid, or if membranes form around liquid vesicles in the midst of coagulated lymph in tissue culture preparations, then lymphatics arise as the result of the pressure exerted by accumulated lakelets of lymph. It is even unjustifiable to transfer to lymphatics the properties of structures which resemble them morphologically so nearly as blood-vessels, for, while there are many points of similarity between the modes of reaction of the two, there are also striking differences.

It is conceivable that future studies may reveal the various stimuli which are responsible for the primary differentiation of tissues and organs. For the lymphatic endothelium it would seem a more hopeful field to investigate the chemical nature of the intercellular fluid, to see whether any evidence can be gained as to the collection there of especial chemical substances which stimulate its differentiation. To propose such an hypothesis at the present time, however, would be pure speculation.

Much confusion has arisen because quite different structures have been grouped together under the name of endothelium or mesothelium. It would seem that the time is ripe to separate these different forms of flattened lining cells under different names. If, for example, we could speak of blood-vessel endothelium as *Haem-angiothelium*, and of lymphatic endothelium as *Lymph-angiothelium*, or some equally specific names, and if distinctive names could be selected for the other forms of pavement epithelium, much of the confusion would disappear.

In conclusion, it is a pleasure to express my gratitude to the Marine Biological Laboratory at Wood's Hole, where these studies were made, for generously granted laboratory facilities.

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AN ANOMALOUS URINOGENITAL SYSTEM IN A DOG

LOUIS H. KORNDER

From the Anatomical Laboratory of the Northwestern University Medical School[1]

TWO FIGURES

Anomalies of the urinogenital system are frequent and have ceased to attract much attention. Few, however, present features of such embryological interest as the following case. Because of this and on account of its value, as illustrating the physiological adaptability of one system to the needs of another, this case seems worthy of mention. My acknowledgment is due Dr. L. B. Arey for valuable suggestions regarding the embryological considerations.

On opening the abdomen of a dog it is commonly observed that the bladder is large and lies almost entirely in the abdominal cavity. In a medium sized mature female, selected at random for the purpose of obtaining certain tissues used in a research problem, the bladder lay deep in the pelvic cavity and was rather small, the size and shape being that of a walnut. On palpation it felt extremely firm, much as though it were a solid mass of tissue. A longitudinal incision through its wall revealed but a very small lumen, less than 1 cm. in diameter and 2 cm. in length.

Two broad ligaments passed from this bladder over the rectum and gained attachment to the front of the sacrum. One ligament was considerably longer than the other, due to the bladder lying ventral and to the left of the uterus instead of directly ventral as is normally the case. Except for these two ligaments and a slightly shortened urethra which merged into the left wall of the urinogenital sinus, other connections with the bladder could not be established.

[1] Contribution No. 40, May 15, 1916.

These findings led to an examination of the kidneys, which were found to be normal in shape, size and position. Each possessed one short ureter, the right being 6.5 cm. and the left 7.2 cm. in length.

Originating in the pelvis of the kidneys the ureters coursed downward over the psoas muscles and passed one on each side into the horns of the bicornuate uterus. This union occurred about a centimeter below the place where the short Fallopian tubes merge into the uterine horns. The uterine horns and the uterus were not soft and pliable as is usual but were hard and rigid and on making a longitudinal incision through their walls, were found to be filled with débris, composed mainly of desquamated epithelial cells.

The structures in this region were surrounded and some deeply imbedded in a mass of fibrous and adipose tissue. This appeared to form a common capsule which covered the union of the ureters with the uterine horns and extended over the Fallopian tubes including the ovaries, becoming at this connection part of the ovarian bursa.

The ovarian bursa exists normally in the dog as a separate fold of peritoneum covering each ovary. This is usually covered by adipose tissue but opens through a small slit-like opening into the abdominal cavity.

It has been mentioned that the urethra was slightly shorter than normal and passed from the left into the wall of the urinogenital sinus. This relation of the urethra to the urinogenital sinus and the original location of the bladder explains why in the accompanying figure (fig. 1) the bladder is shown as lying between the uterus and rectum, instead of ventral to the uterus as is normal.

Histological preparations of the bladder show a slightly changed epithelial lining, consisting in two to three layers of low cuboidal epithelium. The uterine surface epithelium instead of being high columnar in type is pseudo-stratified. The deeper glandular epithelium, however, is the same as in a normal dog's uterus. The ovaries which were deeply imbedded in their ovarian bursae on sectioning showed nothing atypical, with the exception of

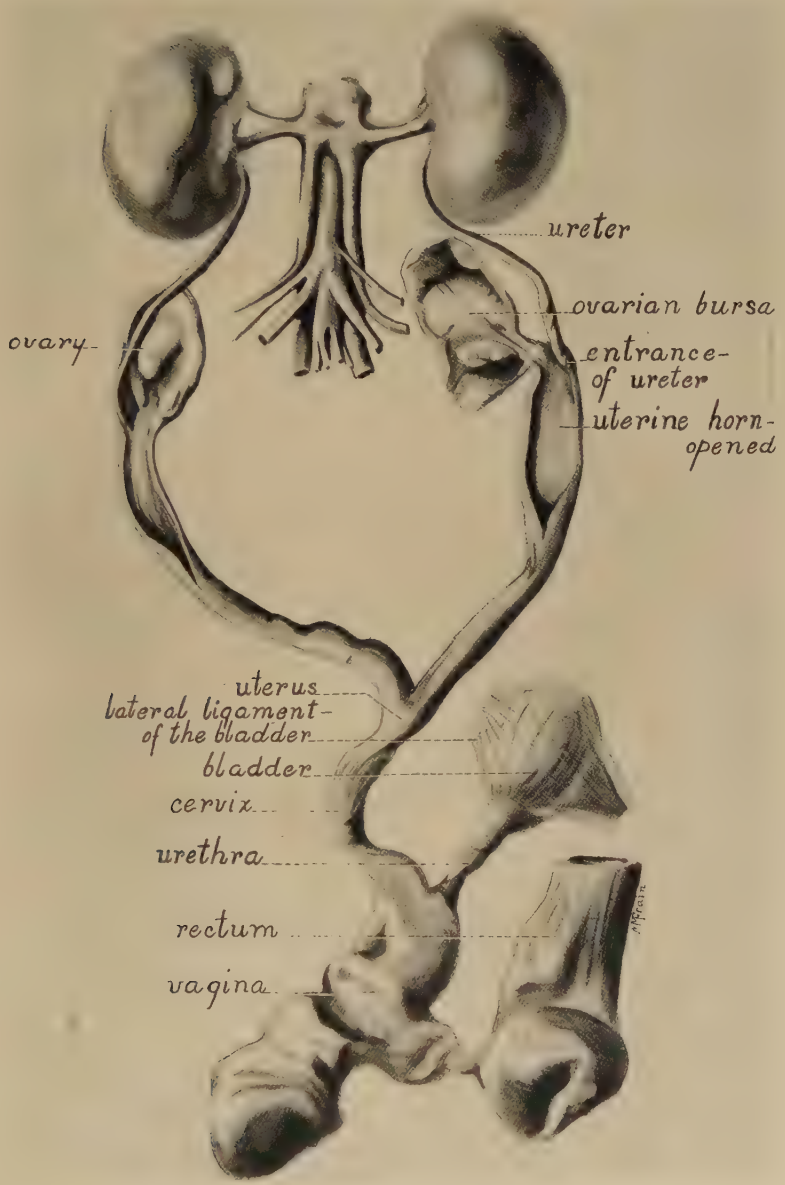

Fig. 1 Ventral view. Ureters shown as they enter both horns of uterus. Ovarian bursae and upper end of uterine horns opened. Bladder small and abnormal in location.

a slightly more fibrous stroma than is usual. Several large Graffian follicles present indicated a normal functional activity of the ovaries. Histologically, then, practically nothing unusual exists, the anomaly being one of gross anatomy, this consisting in a union of the urinary with the reproductive tract, the fusion of ureters and uterine horns leaving the bladder as a cul de sac which leads through the urethra into the urinogenital sinus.

EMBRYOLOGICAL CONSIDERATIONS

The structures involved here are embryological derivatives of the mesonephric ducts, metanephros, Muellerian ducts and cloaca. That the cloaca developed normally is indicated by the presence of a rectum, bladder, urethra and urinogenital sinus. The presence of the uterus, tubes and vagina likewise indicate the normal development of the Muellerian ducts. The anomaly then must be due to a defective embryological growth of the mesonephric ducts and their derivatives, with a deficiency in the development of the nephrogenic cord as a possible causal stimulus.

In pig embryos of approximately 5 mm. length the mesonephric ducts give rise to the ureteric anlage of the metanephros where the ducts bend to join the cloaca. But that the ureteric anlages do not always originate at this bend is indicated by the frequency of double or triple ureters. In these instances the first ureteric bend develops usually into the ureter most normal, while the rest show evidence of slowed or mal-development. From these cases it may be assumed that the ureteric anlage need not necessarily arise at a definite location but can occur at any point along the ducts.

Figure 2, a very diagrammatic sketch, shows both the Muellerian and Wolffian ducts leading into the urinogenital sinus. The approximate position where normally a single metanephric anlage arises from the Wolffian duct is indicated by (*A*). However, in man as many as six such anlages have been observed. While in these instances the anlage corresponding to (*A*) develops into the adult ureter the possibility exists that a more

cranial anlage, for instance (*B*), may become the functional ureter.

Reference to figure 2 will show the lower part of the Wolffian duct not cross-hatched. This portion which extends from the normal ureteric anlage on downwards is during further development drawn into the urinogenital sinus. Through this fusion the ureters receive their normal connection with the definitive bladder which develops partly out of this portion of the sinus.

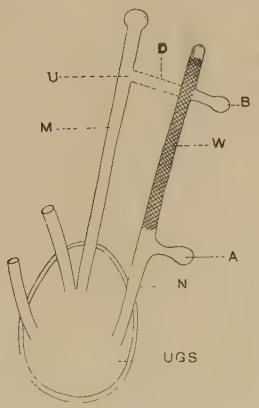

Fig. 2 Diagrammatic sketch. *M*, Muellerian duct; *W*, Wolffian duct; *A*, location of normal metanephric anlage; *B*, possible upwardly displaced metanephric anlage; *D*, extended ureter merging into the Muellerian duct at *U;* N, portion of Wolffian duct taken into wall of urogenital sinus; *UgS.*, cross-hatched portion of Wolffian duct degenerates.

If in this particular case, however, the ureteric anlage did not develop low enough to be included in that lower portion of the mesonephric duct then just as soon as the normal degeneration of the upper part of the Wolffian duct occurred, the upwardly displaced anlage (*B*) which developed into the ureter was without connection with the urinogenital sinus. Being thus isolated it seems probable that the ureter (*D*) extended to the nearby Muellerian duct and merged into it at (*U*). This established an outlet into the urinogenital sinus.

The above is offered as one possibility to which the present anomaly may be due. It is entirely hypothetical as any con-

sideration of this case must be. Because of this a further explanation may possibly be found in the following. In addition to those known embryological facts mentioned above it should be recalled that in embryos of 8 to 11 mm. length the Muellerian ducts develop caudalward beneath the epithelium of the mesonephric fold. Reference to dissections of the pig embryo show the Muellerian ducts lying very close to the Wolffian ducts so that the occurrence of a more or less complete longitudinal fusion of these two ducts seems not impossible.

The establishment of this anomalous union of ureters and uterine horns presumably occurred early in the development of the animal. Since in the female, during the normal development the mesonephric ducts degenerate and disappear almost entirely, it may be assumed that in this case these ducts gradually fused with the Muellerian ducts. It may be that in this way an early connection occurred on either side between the Muellerian duct and an upwardly displaced ureter.

The question may be raised why a metanephros thus displaced should have abandoned the mesonephric duct and appropriated a new outlet by way of the Muellerian ducts? It may be that the functional need of maintaining the patency of the mesonephric duct was ineffectual compared with the tendency toward atrophy and consequent occlusion which the cranial portion normally shows. This query becomes all the more pertinent in view of the recent report by Bremer (Jour. Anat., vol. 19, '16) that in the cat the mesonephros maintains its activity until the permanent kidney assumes the excretory function. This being true of the cat it is more than probable that it also exists in the dog since both belong to the Carnivora.

SENSORY ELEMENTS IN THE HUMAN CEREBRAL HYPOPHYSIS

W. SOHIER BRYANT

ONE FIGURE

In the past few years, the greater part of the work relating to the cerebral hypophysis has been of a therapeutic, a clinical or a surgically experimental nature, and the interest aroused in these aspects of the pituitary has tended to obscure the fact that certain histological elements in the structure of the organ still remain a mystery. The following report re-introduces the subject of the sensory elements of the hypophysial cavity, of which I have made a careful examination in human specimens: These sensory elements occur in maculae, which, in sagittal sections of the pituitary are seen situated on the posterior wall of the cavity, and sometimes, apparently, on the anterior wall. The maculae are composed of tall columnar ciliated sensory cells interspersed with bipolar cells, which have their nuclei towards the periphery; whereas in the ciliated cells, the nuclei are near the base which terminates in a caudal prolongation. Between these caudal processes of the ciliated cells, there is a layer of round cells, resting on a thin basement membrane. An area of ciliated cuboidal cells occurs at the margins of the maculae. I have found these sensory cells in all the freshly hardened human hypophyses that I have examined, except those in which the parenchyma had been almost completely replaced by connective-tissue; the sensory cells are moreover encountered even in pituitaries which have undergone very extensive pathological change. In their gross arrangement, the sensory elements of the hypophyseal cavity are suggestive of the sensory elements of the maculae acousticae.

Gentès (3), in his examination of the hypophysis of cats and dogs, found in the juxta-nervous layer, a stratified cylinder

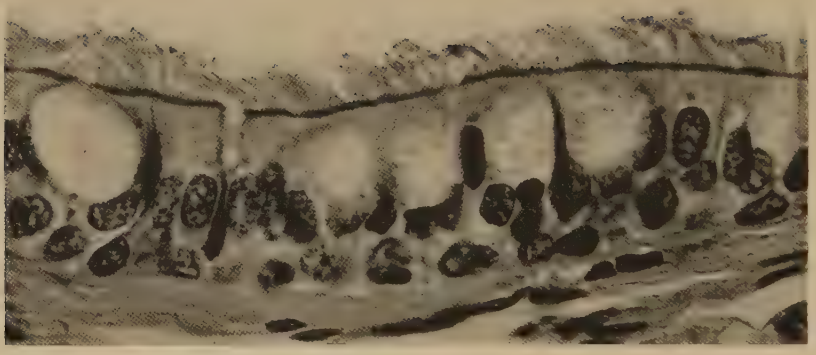

Fig. 1 Sensory epithelium of the hypophysial cavity of a human adult. Stained with hematoxylin-eosin.

This material was procured through the kindness of Dr. William Mabon and the assistance of Dr. Clarence O. Cheney, Pathologist of the Manhattan State Hospital; the work was done in the New York Psychiatric Institute. Special thanks are due Dr. Charles Bates Dunlap for his technical assistance and supervision of the work.

epithelium resembling certain sensory epithelia. Cajal (1) has noted numerous special bi-polar cells in the epithelium adjacent to the nervous lobe in the hypophysis of the rat. Pirone (4), on the basis of his examinations in the cat and the dog, confirms the findings of Gentès: the cylindrical epithelium, in its superficial layers, presents the structure of the supporting cells of sensory epithelia. Gemelli (2) finds that the posterior wall of the hypophysial cavity, in the guinea-pig, is composed of a layer of cylindrical cells, and other cells which he calls supporting cells. The only reference in the literature to sensory elements in the human hypophysis is given by Tello (5), who mentioned that he found sensory epithelium in his human specimens, but omits to give any adequate description of it.

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A METHOD FOR MICRO-INJECTION

EMILY RAY GREGORY

Zoological Laboratory, University of Pennsylvania

ONE FIGURE

After spending several months in an effort to secure satisfactory results and economy of material with methods used by other workers, the writer devised the following apparatus which is very easily arranged and does the work without waste except for accidents due to inexperience or carelessness in handling.

Direct pressure is secured from a good (50c.) De Vilbiss atomizer bulb. The bulb lies on the floor and is kept from rolling by a crocheted net cover. The pressure is transmitted by a red rubber tube $\frac{3}{16}$ of an inch in diameter. After the transmitter reaches the table a short glass tube (h) is inserted in it and a cord (g) run from this to the upright of a small stand with a heavy base to keep these parts in position. Eleven inches further on a glass T (r) is inserted, to the stem of which a thistle tube is joined by a short piece of rubber tubing (p). Eighteen inches more of the transmitter end with a short glass injection tube (c) which has the outer end drawn into a capillary tip at right angles to itself. A sliding rod (i) passed through the pivoted top (b) of the upright of the stand makes a convenient support for the end of the transmitter. A piece of cork (a) on the pointed bent tip of the rod gives enough friction to keep the tube from slipping and still allows it to be readily moved in either direction or removed for use, with one hand. A low Stender dish (d) for the injection fluid and a tumbler of water (b) for cleansing and protecting the injection tube are placed near the stand. A small bull-dog clip completes the essential outfit. This clip placed below the T tube allows the injection tube (c) to be more quickly filled through the capillary tip by applying suction with the mouth through the thistle tube. It is then placed at (p) between the T and the thistle tube when pressure for injection is to be applied.

It will be noticed that this apparatus allows of modifications to suit the worker or to adapt it to special conditions. It does not seem necessary to describe dissecting stands or microscopes but a few words as to the handling of the apparatus and the preparation of certain objects may save time, temper and material for persons attempting injection for the first time.

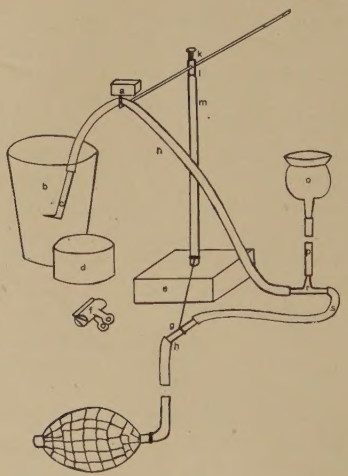
In regard to the apparatus:

1. It is desirable to have a number of injection tubes ready for use. The writer has found it easiest to make these over a fish-tail burner, or a minim burner. Sufficient thin tubing for two tubes is heated at the centre for the shortest possible distance and when sufficiently soft, bent and quickly drawn apart. When one has secured a good tip, it is well to heat the other end enough to smooth it so that it will not cut the rubber tubing on insertion.

2. Remember that capillary tubes are very easily broken. When not in use the injection tube should hang freely in the tumbler where it will be safe from accidents.

3. The tip of the injection tube is very easily clogged. An injection fluid from which there may be any deposit should not be left in the tube a long time. Higgin's waterproof ink will also stand strong dilution with ammoniated water, four drops to the ounce according to their directions. If clogging occurs with the ink, egg albumen or shreds of tissue, to let the tip soak in a strong solution of NaOH often makes it possible to clear it and the NaOH may be easily washed out with water.

4. The clip should be removed when the apparatus is not in use.



To prepare small chick or similar embryos:

Keep the egg in the position in which it is found at the time of use. Remove as much of the albumen as possible by making a small opening on the lower surface and another on the top. The lower opening must be closed as soon as the yolk membrane appears. A piece of paper held in place by one finger will answer. The upper half of the shell may then be removed and the embryo exposed. Irrigation with a warm normal salt solution helps to remove the albumen and so to prevent the clogging of the capillary tube. It is also very desirable to remove the vitelline membrane over the embryonic area if possible. The shell containing the embryo thus prepared, can easily be steadied in a low glass dish with a low raised ring near the centre. Such may sometimes be found at the ten cent stores. A brass curtain ring in a smooth dish would answer the same purpose.

Although the apparatus and method are so simple, it is desirable for any one who has never done this kind of work to practise with inexpensive material in order to gain some facility, before trying to handle that which is rare or costly.